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(54) Title: ANALOGS OF PEPTIDE YY AND USES THEREOF

(57) Abstract

The invention provides analogs of PYY. The invention also provides compositions and methods useful for controlling biological activities such as cell proliferation, nutrient transport, lipolysis, and intestinal water and electrolyte secretion.

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ANALOGS OF PEPTIDE YY AND USES THEREOF

Statement as To Federally Sponsored Research

This invention was made in part with Government funding and the Government therefore has certain rights in the invention.

Background of the Invention

This invention relates to peptide derivatives which are useful as therapeutic agents in the treatment of gastroenterological disorders.

Peptide YY (PYY) is a 36-residue peptide amide isolated originally from porcine intestine, and localized in the endocrine cells of the gastrointestinal tract and pancreas (Tatemoto et al. *Proc. Natl. Acad. Sci.* 79:2514, 1982). Peptide YY has N-terminal and C-terminal tyrosine amides; accordingly, these two tyrosines give PYY its name (Y represents the amino acid tyrosine in the peptide nomenclature). In addition PYY shares a number of central and peripheral regulatory roles with its homologous peptide neuropeptide Y (NPY), which was originally isolated from porcine brain (Tatemoto, *Proc. Natl. Acad. Sci.* 79:5485, 1982). In contrast with the cellular location of PYY, NPY is present in submucous and myenteric neurons which innervate the mucosal and smooth muscle layers, respectively (Ekblad et al. *Neuroscience* 20:169, 1987). Both PYY and NPY are believed to inhibit gut motility and blood flow (Laburthe, *Trends Endocrinol. Metab.* 1:168, 1990), and they are also thought to attenuate basal (Cox et al. *Br. J. Pharmacol.* 101:247, 1990; Cox et al. *J. Physiol.* 398:65, 1988; Cox et al. *Peptides* 12:323, 1991; Friel et al. *Br. J. Pharmacol.* 88:425, 1986) and secretagogue-induced intestinal secretion in rats (Lundberg et al. *Proc. Natl. Acad. Sci USA* 79:4471, 1982; Playford et al. *Lancet* 335:1555, 1990) and humans (Playford et al. *supra*), as well as stimulate

net absorption (MacFadyen et al. *Neuropeptides* 7:219, 1986). Furthermore, plasma PYY levels have been reported to be elevated in several diseases that cause diarrhea (Adrian et al. *Gastroenterology* 89:1070, 1985). Taken together, these observations suggest that PYY and-NPY are released into the circulation after a meal (Adrian et al. *Gastroenterology* 89:1070, 1985; Balasubramaniam et al. *Neuropeptides* 14:209, 1989), and thus may play a physiological role in regulating intestinal secretion and absorption, serving as natural inhibitors of diarrhea.

A high affinity PYY receptor system which exhibits a slightly higher affinity for PYY than NPY has been characterized in rat intestinal epithelia (Laburthe et al. *Endocrinology* 118:1910, 1986; Laburthe, *Trends Endocrinol. Metab. supra*) and shown to be negatively coupled to adenylate cyclase (Servin et al. *Endocrinology* 124:692, 1989). Consistently, PYY exhibited greater antisecretory potency than NPY in voltage clamped preparations of rat small intestine (Cox et al. *J. Physiol. supra*), while C-terminal fragments of NPY were found to be less effective in their antisecretory potency than PYY (Cox et al. *Br. J. Pharmacol. supra*). Structure-activity studies using several partial sequences have led to the identification of PYY(22-36) as the active site for interacting with intestinal PYY receptors (Balsubramaniam et al. *Pept. Res.* 1:32, 1988).

In addition, PYY has been implicated in a number of physiological activities including nutrient uptake (see, e.g., Bilcheik et al. *Digestive Disease Week* 506:623, 1993), cell proliferation (see, e.g., Laburthe, *Trends Endocrinol. Metab.* 1:168, 1990; Voisin et al. *J. Biol. Chem*, 1993), lipolysis (see, e.g., Valet et al., *J. Clin. Invest.* 85:291, 1990), and vasoconstriction

(see, e.g., Lundberg et al., *Proc. Natl. Acad. SCi., USA* 79: 4471, 1982).

The amino acid sequences of porcine and human PYY are as follows:

porcine PYY YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY (SEQ. ID. NO. 1)

human PYY YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY (SEQ. ID. NO. 2)

The amino acid sequence for dog PYY and rat is the same as porcine PYY.

Summary of the Invention

In one aspect, the present invention features novel analogs of peptide YY of the formula:

$$\begin{array}{l} R_1 \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad R_3 \\ \;\;\diagdown \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\;\; \diagup \\ R_2\text{-}X\text{-}A^{22}\text{-}A^{23}\text{-}A^{24}\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}Y\text{-}R_4 \end{array}$$

wherein

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R_1 and R_2;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to R_3 and R_4;

R_1 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, naphthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

R_2 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl,naphthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g.,benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^{22} is an aromatic amino acid, Ala,

Aib, Anb, N-Me-Ala, or is deleted;

A^{23} is Ser, Thr, Ala, Aib, N-Me-Ser, N-Me-Thr, N Me-Ala, or is deleted;

A^{24} is Leu, Ile, Val, Trp, Gly, Aib, Anb, N-Me-Leu, or is deleted;

A^{25} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, or is deleted;

A^{26} is Ala, His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, or is deleted;

A^{27} is an aromatic amino acid other than Tyr;

A^{28} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{29} is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A^{30} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{31} is Val, Ile, Trp, Aib, Anb, or N-Me-Val;

A^{32} is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;

R_3 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, naphthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl); and

R_4 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, naphthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl),

or a pharmaceutically acceptable salt thereof.

In preferred embodiments, A^{27} is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

In preferred embodiments X is $A^{17}-A^{18}-A^{19}-A^{20}-A^{21}$ wherein

A^{17} is Cys, Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{18} is Cys, Ser, Thr, N-Me-Ser, or N-Me-Thr;

A^{19} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), Cys, or Orn;

A^{20} is an aromatic amino acid, or Cys; and

A^{21} is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof. In yet other preferred embodiments, Y is $A^{33}-A^{34}-A^{35}-A^{36}$ wherein

A^{33} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Cys, or Orn;

A^{34} is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Aib, or Anb;

A^{35} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R(where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Cys, or Orn; and

A^{36} is an aromatic amino acid, Cys or a pharmaceutically acceptable salt thereof.

Preferably, the compound has the formula: N-α-Ac-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 3), H-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 4), N-α-Ac-Ala-Ser-Leu-Arg-His-Trp-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 5), N-α-Ac-Ala-Ser-Leu-Arg-His-Thi-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 6), N-α-Ac-Tyr-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 7) or a pharmaceutically acceptable salt thereof.

In another aspect the invention features novel analogs of peptide YY of the formula:

$$
\begin{array}{l}
R_1 \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad R_3 \\
\quad\backslash \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad / \\
R_2\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}Y\text{-}R_4
\end{array}
$$

wherein

the N-terminal amino acid is bonded to R_1 and R_2;

Y is a chain of 0-4 amino acids, inclusive the C-terminal one of which is bonded to R_3 and R_4;

R_1 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, napthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

R_2 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, napthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^{25} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, or is deleted;

A^{26} is Ala, His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn, or is deleted;

A^{27} is an aromatic amino acid;

A^{28} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{29} is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A^{30} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{31} is Val, Ile, Trp, Aib, Anb, or N-Me-Val;

A^{32} is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;

R_3 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, napthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl); and

R_4 is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl, napthaleneacetyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl), or a pharmaceutically acceptable salt thereof.

In preferred embodiments A^{27} is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

In preferred embodiments Y is A^{33}-A^{34}-A^{35}-A^{36} wherein

A^{33} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), Cys, or Orn;

A^{34} is Gln, Asn, Ala, Gly, N-Me-Gln, Aib, Cys, or Anb;

A^{35} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), Cys, or Orn; and

A^{36} is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof. Preferably, the compound has the formula N-α-Ac-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 8).

In another aspect, the invention features novel dimeric analogs of peptide YY. The dimer may be formed by either including two peptides of Formula I, two peptides of Formula II, or one peptide of Formula I and one peptide of Formula II. In one embodiment, the dimer

is formed by utilizing a dicarboxylic acid linker capable of binding to a free amine, either primary or secondary, located within each peptide. See, e.g., R. Vavrek and J. Stewart, Peptides: Structure and Function 381-384 (Pierce Chemical Co. 1983). Examples of suitable dicarboxylic acid linkers are succinic acid, glutamic acid, and phthalic acid. In other embodiments, the dimer is formed by utilizing an amino acid linker capable of binding to a free amine group of one peptide and a free carboxyl group of the other peptide. Preferably, the amino acid linker is a non α-amino acid. Examples of suitable amino acid linkers are amino-caproic acid and amino-valeric acid. In yet another embodiment, the dimer is formed by a disulfide bridge between cysteines located within each peptide. See, e.g., M. Berngtowicz and G. Piatsueda, Peptides: Structure and Function 233-244 (Pierce Chemical Co. 1985); F. Albericio, et al., Peptides 1990. 535 (ESCOM 1991).

The symbol X, Y, Z; A^{22}, A^{23}, A^{24}, and the like; and Ser, Leu or the like, as found in a peptide sequence herein stands for an amino acid residue, i.e., =N-CH(R)-CO- when it is at the N-terminus, or -NH-CH(R)-CO-N= when it is at C-terminus, or -NH-CH(R)-CO- when it is not at the N- or C-terminus, where R denotes the side chain (or identifying group) of an amino acid or its residue. For example, R is $-CH_2COOH$ for Asp, R is -H for Gly, R is $-CH_2OH$ for Ser, R is $-CH_3$ for Ala and R is $-CH_2CH_2CH_2CH_2NH_2$ for Arg. Also, when the amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated.

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art;

but for clarity are listed below. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a peptide bond.

Asp	D	Aspartic Acid
Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Cys	C	Cysteine
Gly	G	Glycine
Glu	E	Glutamic Acid
Gln	Q	Glutamine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine

Orn = Ornithine
Nal = 2-napthylalanine
Thi = 2-thienylalanine
Pcp = 4-chlorophenylalanine
Bth = 3-benzothienyalanine
Bip = 4,4'-biphenylalanine
Tic = tetrahydroisoquinoline-3-carboxylic acid

Aib = aminoisobutyric acid

Anb = α-aminonormalbutyric acid

Dip = 2,2-diphenylalanine

Thz = 4-Thiazolylalanine

The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids, such as hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid and the like.

In another aspect, the invention features one of the above compounds and a pharmaceutically acceptable carrier substance in a therapeutic composition capable of decreasing excess intestinal water and electrolyte secretion.

In preferred embodiments, the composition is in the form of a liquid, pill, tablet, or capsule for oral administration; a liquid capable of being administered nasally as drops or spray or a liquid for intravenous, subcutaneous, parenteral, intraperitoneal or rectal administration. The therapeutic composition can also be in the form of an oil emulsion or dispersion in conjunction with a lipophilic salt such as pamoic acid, or in the form of a biodegradable sustained-release composition for subcutaneous or intramuscular administration. For maximum efficacy, zero-order release is desired.

In another aspect the invention features, a method for decreasing excess intestinal water and electrolyte secretion in a mammal, the method comprising

administering to the mammal, e.g., a human, a therapeutically effective amount of the above mentioned compounds.

In addition, the invention features a method of regulating cell proliferation in a mammal, the method comprising administering to the mammal a therapeutically effective amount of the composition of the above mentioned compounds. Preferably, the method regulates the proliferation of an intestinal cell.

The invention also features methods for increasing nutrient transport, regulating lipolysis, and regulating blood flow in a mammal, the methods comprising administering to the mammal a therapeutically effective amount of the above mentioned compositions.

The compounds of the invention exhibit a broad range of biological activities related to their antisecretory and antimotility properties. The compounds are believed to suppress gastrointestinal secretions by direct interaction with epithelial cells or, perhaps, by inhibiting secretion of hormones or neurotransmitters which stimulate intestinal secretion. The compounds of the invention may also control intestinal blood flow which in turn may modulate intestinal hydrostatic pressure in favor of net water absorption.

The compounds of the invention are especially useful in the treatment of any number of gastrointestinal disorders (see e.g., *Harrison's Principles of Internal Medicine*, McGraw-Hill Inc., New York, 12th Ed.) that are associated with excess intestinal electrolyte and water secretion as well as decreased absorption, e.g., infectious (e.g., viral or bacterial) diarrhea, inflammatory diarrhea, short bowel syndrome, or the diarrhea which typically occurs following surgical procedures, e.g., ileostomy. Examples of infectious diarrhea include, without limitation, acute viral

diarrhea, acute bacterial diarrhea (e.g., salmonella, campylobacter, and clostridium or due to protozoal infections), or traveller's diarrhea (e.g., Norwalk virus or rotavirus). Examples of inflammatory diarrhea include, without limitation, malabsorption syndrome, tropical spue, chronic pancreatitis, Crohn's disease, diarrhea, and irritable bowel syndrome. It has also been discovered that the peptides of the invention can be used to treat an emergency or life-threatening situation involving a gastrointestinal disorder, e.g., after surgery or due to cholera. Furthermore, the compounds of the invention can be used to treat patients suffering from Acquired Immune Deficiency Syndrome (AIDS), especially during cachexia.

The compounds of the invention are also useful for inhibiting small intestinal fluid and electrolyte secretion, augmenting nutrient transport -- as well as increasing cell proliferation -- in the gastrointestinal tract, regulating lipolysis in, e.g, adipose tissue, and regulating blood flow in a mammal.

The compounds of the invention are advantageous because they are truncated versions of the natural PYY peptide; thus, the shorter peptide not only facilitates easier synthesis and purification of the compounds, but also improves and reduces manufacturing procedures and expenses. Moreover, a shorter PYY compound is advantageous because such peptides will interact solely with PYY receptors and not with homologous receptors such as NPY Y1 and Y3; thus, minimizing unwanted agonist or antagonist side reactions.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings will first be described.

Drawings

FIG. 1 shows a semipreparative reversed phase chromatogram of N-α-Ac-[Phe^{27}]PYY(22-36) (SEQ. ID. NO. 3) (≈25mg) obtained by HF cleavage. Conditions: Vydac C18 semipreparative column (250 X 10mm, 300 Å pore size, 10 micron particle size); flow rate 4.7 ml/min; fractions 1, 2, 3, and 4 were collected and analyzed by analytical chromatography. The homogeneous fractions (1-3) were combined and dried in a speed vac.

FIG. 2 shows a graph of the inhibition of ^{125}I-PYY binding to rat jejunal membranes by increasing concentrations of PYY (SEQ. ID. NO. 1), PYY(22-36) (SEQ. ID. NO. 10), [Im-DNP-His^{26}]PYY (SEQ. ID. NO. 9), [Ala^{32}]PYY(22-36) (SEQ. ID. NO. 11), [Ala^{23,32}]PYY(22-36) (SEQ. ID. NO. 12), [Glu^{28}]PYY(22-36) (SEQ. ID. NO. 13), N-α-Ac-PYY(22-36) (SEQ. ID. NO. 14), N-α-Ac-[p.Cl-Phe^{28}]PYY(22-36) (SEQ. ID. NO. 15), N-α-Ac-[Glu^{26}]PYY(22-36) (SEQ. ID. NO. 16), N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3), N-α-Ac-[N-Me-Tyr^{26}]PYY(22-36)(SEQ. ID. NO. 17), N-α-Myristoyl-PYY(22-36)(SEQ. ID. NO. 18), N-α-Naphthaleneacetyl-PYY(22-36)(SEQ. ID. NO. 19), and PYY (22-26)(SEQ. ID. NO. 10).

FIGS. 3A-B show the antisecretory effects of PYY (SEQ. ID. NO. 1), PYY(22-36)(SEQ. ID. NO. 10) and analogs up one baseline short circuit current (SCC) in voltage clamped preparation of rat jejunum. Values of changes in SCC are quoted of μA/0.6cm^2, mean ±SEM from between 3 and 7 different jejunal preparations. Peptides shown in A and B are denoted by the same symbol as in FIG. 2.

FIG. 4 shows a graph of the inhibition of ^{125}I-PYY binding to rat jejunal membranes by increasing concentrations of PYY, N-α-Ac-PYY(22-36) (SEQ. ID. NO. 14), N-α-Ac-[Tic^{27}]PYY(22-36)(SEQ. ID. NO. 25), N-α-Ac-

[Bip^{27}]PYY(22-36)(SEQ. ID. NO. 22), N-α-Ac-[Nal^{27}]PYY(22-36)(SEQ. ID. NO. 23), N-α-Ac-[Bth^{27}]PYY(22-36)(SEQ. ID. NO. 21), N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3), N-α-Ac-[Phe^{27}]PYY(25-36)(SEQ. ID. NO. 26), N-α-Ac-[Trp^{27}]PYY(22-36)(SEQ. ID. NO. 5), and N-α-Ac-[Thi^{27}]PYY(22-36)(SEQ. ID. NO. 6).

There now follows a description of the synthesis, analysis for biological efficacy and use of the preferred embodiments of the invention. In order to determine the structural requirements necessary to elicit antisecretory effects, several analogs of the PYY active site, PYY(22-36), were synthesized and their binding and antisecretory potencies in rat jejunum were compared.

We now describe the structure, synthesis, and use of preferred embodiments of the invention.

STRUCTURE

The peptides of the invention have the general formula recited in the Summary of the Invention above. They all have an aromatic amino acid group at position 27 which is important for both antisecretory activity and utility as antidiarrheal compounds.

SYNTHESIS

The peptides of the present invention may be synthesized by any techniques that are known to those skilled in the peptide art. An excellent summary of the many techniques so available may be found in *Solid Phase Peptide Synthesis* 2nd ed. (Stewart, J.M. and Young, J. D. Pierce Chemical Company, Rockford, IL, 1984).

The peptides listed in Table 1 and Table 2 were synthesized as follows. Peptide synthesis was performed on an Applied Biosystems Model 430A synthesizer. Amino acid and sequence analyses were carried out using Waters Pico-Tag and Applied Biosystems Model 470A instruments,

respectively. Peptides were purified using a Waters Model 600 solvent delivery system equipped with a Model 481 Spectrophotometer and U6K injector according to standard protocols. Peptide masses were determined at the University of Michigan, Protein Chemistry Facility, Ann Arbor, Michigan according to standard methods. All Boc-L-amino acid derivatives, solvents, chemicals and the resins were obtained commercially and used without further purification.

Paramethylbenzhydroxylamine (MBHA) resin (0.45 mmol, $-NH_2$) was placed in the reaction vessel of the peptide synthesizer and the protected amino acid derivatives were sequentially coupled using the program provided by the manufacturers modified to incorporate a double coupling procedure (see, e.g., Balasubramaniam et al., *Peptide Research* 1: 32, 1988). All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Gln and Asn, however, were coupled as preformed 1-hydroxybenzotriazole (HOBT) esters to avoid side reactions. At the end of the synthesis, the N-α-Boc group was removed and in some instances the free α-NH2 was acetylated by reaction with acetic anhydride (2 equivalents) and diisopropyl ethylamine until a negative ninhydrin test was obtained (Anal. Biochem. 34:595, 1970). The peptide resin (~1.0 g) was then treated with HF (10 ml) containing p-cresol (~0.8 g) for 1 h at -2 to -4 °C. The HF was evacuated and the residue was transferred to a fritted filter funnel with diethyl ether, washed repeatedly with diethyl ether, extracted with acetic acid (2 X 15 ml) and lyophilized. The crude peptides thus obtained were purified by semipreparative RP-HPLC as shown in Fig. 1.

Examples of the synthesized nalogs are:

[im-DNP-His^{26}]PYY

YPAKPEAPGEDASPEELSRYYASLR [im-DNP-His^{26}]YLNLVTRQRY-NH_2 (SEQ. ID No. 9)

PYY(22-36)

A S L R H Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 10)

[Ala^{32}]PYY

A S L R H Y L N L V [Ala] R Q R Y-NH_2 (SEQ. ID No. 11)

[Ala^{23,32}]PYY

A [Ala] L R H Y L N L V [Ala] R Q R Y-NH_2 (SEQ. ID No. 12)

[Glu^{28}]PYY(22-36)

A S L R H Y [Glu] N L V T R Q R Y-NH_2 (SEQ. ID No. 13)

N-α-Ac-PYY(22-36)

N-α-Ac-A S L R H Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 14)

N-α-Ac[p.Cl.Phe^{26}]PYY

N-α-Ac-A S L R [p.Cl.Phe^{26}] Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 15)

N-α-Ac[Glu^{28}]PYY

N-α-Ac-A S L R H Y [Glu] N L V T R Q R Y-NH_2 (SEQ. ID No. 16)

N-α-Ac[Phe^{27}]PYY

N-α-Ac-A S L R H [Phe] E N L V T R Q R [N-Me-Tyr]-NH_2 (SEQ. ID No. 3)

N-α-Ac[N-Me-Tyr^{36}]PYY

N-α-Ac-A S L R H Y E N L V T R Q R [N-Me-Tyr]-NH_2 (SEQ. ID No. 17)

N-α-myristoyl-PYY(22-36)

N-α-myristoyl-A S L R H Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 18)

N-α-naphthaleneacetyl-PYY(22-36)

N-α-naphthaleneacetyl -A S L R H Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 19)

N-α-Ac[Phe^{27}]PYY

N-α-Ac-A S L R H [Phe] E N L V T R Q R [N-Me-Tyr]-NH_2 (SEQ. ID No. 3)

N-α-Ac-PYY(22-36)

N-α-Ac-A S L R H Y L N L V T R Q R Y-NH_2 (SEQ. ID No. 20)

N-α-Ac-[Bth^{27}]PYY(22-36)

N-α-Ac-A S L R H [Bth] L N L V T R Q R Y-NH_2 (SEQ. ID No. 21)

N-α-Ac-[Bip^{27}]PYY(22-36)

N-α-Ac-A S L R H [Bip] L N L V T R Q R Y-NH_2 (SEQ. ID No. 22)

N-α-Ac-[Nal^{27}]PYY(22-36)

N-α-Ac-A S L R H [Nal] L N L V T R Q R Y-NH_2 (SEQ. ID No. 23)

N-α-Ac-[Trp^{27}]PYY(22-36)

N-α-Ac-A S L R H [Trp] L N L V T R Q R Y-NH_2 (SEQ. ID No. 5)

N-α-Ac-[Thi^{27}]PYY(22-36)

N-α-Ac-A S L R H [Thi] L N L V T R Q R Y-NH_2 (SEQ. ID No. 6)

N-α-Ac-[Tic^{27}]PYY(22-36)

N-α-Ac-A S L R H [Tic] L N L V T R Q R Y-NH_2 (SEQ. ID No. 25)

N-α-Ac-[Phe^{27}]PYY(25-36)

N-α-Ac-H [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 26)

N-α-Ac-[Phe^{27},Thi^{36}]PYY(22-36)

N-α-Ac-A S L R H [Phe] L N L V T R Q R [Thi]-NH_2 (SEQ. ID No. 27)

N-α-Ac-[Thz^{26},Phe^{27}]PYY(22-36)

N-α-Ac-A S L R [Thz] [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 28)

N-α-Ac-[Pcp^{27}]PYY(22-36)

N-α-Ac-A S L R N [Pcp] L N L V T R Q R Y-NH_2 (SEQ. ID No. 29)

N-α-Ac-[$Phe^{22,27}$]PYY(22-36)
N-α-Ac-[Phe] S L R H [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 30)

N-α-Ac-[Tyr^{22},Phe^{27}]PYY(22-36)
N-α-Ac-[Tyr] S L R H [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 7)

N-α-Ac-[Trp^{28}]PYY(22-36)
N-α-Ac- A S L R H Y [Trp] N L V T R Q R Y-NH_2 (SEQ. ID No. 31)

N-α-Ac-[Trp^{30}]PYY(22-36)
N-α-Ac- A S L R H Y L N [Trp] V T R Q R Y-NH_2 (SEQ. ID No. 32)

N-α-Ac-[Ala^{26},Phe^{27}]PYY(22-36)
N-α-Ac- A S L R [Ala] [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 33)

N-α-Ac-[Bth^{27}]PYY(22-36)
N-α-Ac- A S L R N [Bth] L N L V T R Q R Y-NH_2 (SEQ. ID No. 34)

N-α-Ac-[Phe^{27}]PYY(22-36)
N-α-Ac- A S L R N [Phe] L N L V T R Q R Y-NH_2 (SEQ. ID No. 35)

N-α-Ac-[$Phe^{27,36}$]PYY(22-36)
N-α-Ac- A S L R N [Phe] L N L V T R Q R [Phe]-NH_2 (SEQ. ID No. 36)

N-α-Ac-[Phe^{27}, D-Trp^{32}]PYY(22-36)
N-α-Ac- A S L R N [Phe] L N L V [D-Trp] R Q R Y-NH_2 (SEQ. ID No. 37)

ANALYSIS

Binding Studies

Preparation of ^{125}I-PYY labeled only at Tyr^{36} and rat jejunal epithelial plasma membranes were performed according to standard methods (see, e.g., Laburthe et al. *Endocrinology, supra;* Servin et al. *supra;* Voisin et al. *Ann. N. Y. Acad. Sci.* 611:343, 1990). Binding experiments were conducted in a total volume of 0.25 ml 60 mM HEPES buffer, pH 7, containing 2% BSA, 0.1% bacitracin, 5 mM $MgCl_2$ and 0.05 nM ^{125}I-PYY with or without competing peptides. Bound and free peptides were separated by centrifugation at 20,000 X *g* for 10 min. Non-specific ^{125}I-PYY binding was determined in the presence of 1 μM unlabeled PYY represented 10% of the total binding.

Short Circuit Current Measurements

The antisecretory effects of the peptides were investigated by measuring the short-circuit current (SCC) in rat jejunal mucosa mounted in a Ussing chamber and

automatically voltage clamped as described by Cox et al. (*J. Physiol. supra*). Briefly, strips of mucosa were placed between two halves of perspex Ussing chambers (window size, 0.6 cm^2) containing oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution (NaCl, 117 mM, KCl 4.7 mM, $CaCl_2$, 2.5 mM; $MgSO_4$ 1.2 mM, $NaHCO_3$ 24.8 mM and glucose 11.1 mM), pH 7.4, 37°C. Routinely, four preparations of jejunum were obtained from each animal and these exhibited comparable potential differences and SCC, but they were not paired. Preparations were automatically voltage clamped using a W-P dual voltage clamp and the SCC displayed continuously on pen recorders. Once a stable baseline SCC was reached, peptides were added to the basolateral reservoir only, and cumulative concentration-response profiles constructed.

Data Analyses

All points in the binding experiments are the mean of at least three experiments performed in duplicate. For clarity, the SEMs in the binding experiments are not shown in Fig. 2, but were less than 10%. Values of changes in SCC are quoted as $\mu A/0.6cm^2$ mean ± 1 SEM from between 3 and 7 different preparations. EC_{50} values were calculated from pooled cumulative concentration - response curves using an iterative curve fitting program. Comparison of data groups (SCC recordings) were made using unpaired Student's t-tests where a p value <0.5 was considered statistically significant.

There now follows the results of the biological activities of the compounds of the invention (see Table 1 and Table 2). As described below, the tested compounds were assayed for purity and for their binding and antisecretory potencies in rat jejunum.

Purified peptides were found to be > 96% homogeneous by analytical reversed phase chromatography and, in addition, had the expected amino acid composition

and masses. For example, Fig. 1 shows the RP-HPLC chromatogram of N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3). The free peptides were further characterized by sequence analysis (see, Table 1 and Table 2). The overall yields of the peptides were in the range of 10% to 30%.

PYY,[im-DNP-His^{26}]PYY (SEQ. ID. NO. 9) and the analogs of PYY(22-36)(SEQ. ID. NO. 10) displaced ^{125}I-PYY bound to rat jejunal epithelial plasma membranes in a concentration-dependent manner. Although [im-DNP-His^{26}]PYY (SEQ. ID. NO. 9) and PYY(22-36) (SEQ. ID. NO. 10) were 20-times less potent than PYY based on IC_{50} values, they displayed the same maximal response as the intact hormone (Fig. 2, Table 1). Substitution of Thr^{32} with Ala as in [Ala^{32}]PYY(22-36)(SEQ. ID. NO. 11) resulted in the lowering of the binding potency while the replacement of both Ser^{23} and Thr^{32} with Ala further reduced the receptor affinity. Also, introduction of a negative charge at position 28 without altering the helicity as in [Glu^{28}]PYY(22-36)(SEQ. ID. NO. 13) decreased the binding possibly due to the disruption of the ionic interactions. Since the hydrophobic groups are known to increase the interaction with the receptors (Balasubramaniam et al. *Biochem. Biophys. Res. Comm.* 137:1041, 1986), the binding of a N-α-myristoyl- and N-α-naphthaleneacetyl-derivatives of PYY(22-36) was also determined. Both these analogs exhibited slightly lower binding affinity than PYY(22-36)(SEQ. ID. NO. 10) possibly due to increased steric hinderance. On the other hand, N-α-acetylation of PYY(22-36) (SEQ. ID. NO. 14) increased the receptor affinity four times. Further structure-activity studies with N-α-Ac-PYY(22-36) (SEQ. ID. NO. 20) revealed that substitution of Tyr^{36} with N-Me-Tyr or His^{26} with p.Cl-Phe lowers the binding potency. However, replacement of Tyr^{27} with Phe increased the receptor affinity by 28%. As a control, the binding of

PYY(22-36)(SEQ. ID. NO. 10) and several of its analogs were also tested. However, none of these analogs inhibited the binding of ^{125}I-PYY even at 10 μM.

In mucosal preparations of rat jejunum PYY(22-36) (SEQ. ID. NO. 10) analogs reduced the baseline SCC in a concentration dependent manner (Fig. 3A and B) and calculated EC_{50} values are listed in Table 1. The PYY(22-36) (SEQ. ID. NO. 10) analogs were generally less potent as antisecretory agents than as inhibitors of binding. The order of analog potency was similar to that from binding studies with two notable exceptions, namely N-α-myristoyl-PYY(22-36) (SEQ. ID. NO. 18) and N-α-naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19). N-α-acetylation and substitution of Tyr^{27} with Phe increased the antisecretory potency of PYY(22-36) and this analog, N-α-Ac-[Phe^{27}] PYY(22-36) (SEQ. ID. NO. 3), was only 9-times less potent than the intact hormone. Furthermore, there was no significant difference between the maximal inhibitory responses, these being - 12.6±2.4 and - 12.0±1.3μ A/0.6cm^2 for PYY (440 nM, n = 6) (SEQ. ID. NO. 1) and N-α-Ac-[Phe^{27}] PYY(22-36) (1.4 μM, n = 7) (SEQ. ID. NO. 3), respectively.

TABLE 1: Comparison of the binding and antisecretory potencies of PYY, PYY fragments and their analogs

PEPTIDES	RT[a] (min)	MH+ (Calc.)	BINDING[b] IC_{50}(nM)	SCC[b] EC_{50}(nM)
PYY (SEQ. ID. NO. 1)	4.8	4240.2 (4241.7)	0.2	1.7
NPY (SEQ. ID. NO. 24)	34.0[c]	4253.8 (4254.7)	2.0	9[d]
[im-DNP-His^{26}]PYY (SEQ. ID. NO. 9)	8.7[c]	4406.9 (4407.8)	4.0	72
PYY(22-36) (SEQ. ID. NO. 10)	4.4	1888.8 (1890.2)	4.0	77
[Ala^{32}]PYY(22-36) (SEQ. ID. NO. 11)	4.7	1858.8 (1860.2)	71	n.d.
[Ala^{23,32}]PYY(22-36) (SEQ. ID. NO. 12)	4.3	1842.8 (1844.2)	>10,000	n.d.
[Glu^{28}]PYY(22-36) (SEQ. ID. NO. 13)	3.8	1905.1 (1906.2)	199	n.d.
N-α-Ac-PYY(22-36) (SEQ. ID. NO. 14)	10.0	1930.9 (1932.2)	1.12	40
N-α-Ac-[p.ClPhe^{26}]PYY(22-36) (SEQ. ID. NO. 15)	14.9[c]	1975.4 (1976.7)	50	124
N-α-Ac-[Glu^{28}]PYY(22-36) (SEQ. ID. NO. 16)	3.9	1947.0 (1948.2)	44.7	3,000
N-α-Ac-[N-Me-Tyr^{36}]PYY(22-36) (SEQ. ID. NO. 17)	13.5	1945.3 (1946.3)	354	792
N-α-Ac-[Phe^{27}]PYY(22-36) (SEQ. ID. NO. 3)	8.3	1915.3 (1916.2)	0.80	15.1
N-α-Myristoyl-PYY(22-36) (SEQ. ID. NO. 18)	4.8	2099.0 (2100.6)	17.8	3,300
N-α-Naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19)	17.0	2056.9 (2058.4)	8.9	19,500

a: isocratic, 27% CH_3CN containing 0.1% TFA; b: mean of three separate experiments;
c: isocratic, 32% CH_3CN containing 0.1% TFA; d: from reference 10; n.d.: not determined

N-α-myristoyl-PYY(22-36)(SEQ. ID. NO. 18) and N-α-naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19) analogs, in contrast to their moderate binding potency, exhibited poor antisecretory responses with threshold concentrations of about 20nM and EC_{50} values greater than 2 and 30 μM respectively. After a cumulative concentration of 7.4 μM, N-α-myristoyl-PYY(22-36) (SEQ. ID. NO. 18) reduced the basal SCC by - 5.2±0.6μA/0.6cm^2 (n = 7). Subsequent addition of PYY (100 nM) further reduced the SCC by -10.2±0.7μA/0.6cm^2 (n = 7) and this was not significantly different from control responses to PYY(22-36) (SEQ. ID. NO. 10) could antagonize PYY responses, three tissues were treated with the analog (1μM) and PYY concentration-response curves were constructed and compared with controls. The fragment reduced the basal current by -0.4±0.3 μA/0.6cm^2 and the resultant PYY EC_{50} value (4.4±1.2 nM, n = 3) did not differ significantly from that of the nontreated controls (2.6±1.1 nM, n = 3).

These results show that modification of the active site of PYY (SEQ. ID. NO. 1), PYY(22-36)(SEQ. ID. NO. 10), can lead to a substantial increase in both the binding and antisecretory potencies of this fragment. The key analogs in this series exhibited the following order of potency: PYY (SEQ. ID. NO. 1) > N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3) > N-α-Ac-PYY(22-36)(SEQ. ID. NO. 14) > PYY(22-36) (SEQ. ID. NO. 10). Furthermore, our investigations revealed that the hydroxyl groups of Ser^{23} and Thr^{32} as well as the imidazole group of His^{26} are important for interaction with intestinal PYY-preferring receptors. Although there was, in general, a good correlation between the binding and antisecretory potencies of the analogs, there were also notable exceptions.

N-α-myristoyl-PYY(22-36)(SEQ. ID. NO. 18) and N-α-naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19) analogs inhibited ^{125}I-PYY binding with moderate potency, but exhibited poor antisecretory responses. This observation suggested that these analogs may be antagonists. However, prior pretreatment of jejunal membranes with these analogs failed to significantly alter the antisecretory responses to PYY and the reason for the discrepancy remains unclear at present.

Table 2 and Fig. 4 present the IC_{50} values for additonal PYY(22-36) (SEQ. ID. NO. 10) and PYY (25-36) analogs. Based on the results presented in Table 2 the analogs in this series exhibited the following order of potency:

N-α-Ac-[Tic^{27}]PYY(22-36)(SEQ. ID. NO. 25) < N-α-Ac-[Bip^{27}]PYY(22-36) (SEQ. ID. NO. 22) < N-α-Ac-[Nal^{27}]PYY(22-36) (SEQ. ID. NO. 23) < N-α-Ac-[Bth^{27}]PYY(22-36) (SEQ. ID. NO. 21) < N-α-Ac-[Phe^{27}]PYY(22-36) (SEQ. ID. NO. 3) < N-α-Ac-[Phe^{27}]PYY(25-36) (SEQ. ID. NO. 26) < N-α-Ac-[Trp^{27}]PYY(22-36) (SEQ. ID. NO. 5) < N-α-Ac-[Thi^{27}]PYY(22-36) (SEQ. ID. NO. 6) < N-α-Ac-PYY(22-36)(SEQ. ID. NO. 14) < PYY (SEQ. ID. NO. 1).

TABLE 2 Comparison of Receptor Binding Data for PYY and PYY analogs

PEPTIDE NO.	Peptide Structure	IC_{50} (nM)
	PYY (SEQ. ID. NO. 1)	0.04
	N-α-Ac-PYY(22-36) (SEQ. ID. NO. 14)	0.08
905	N-α-Ac-[Bth^{27}]PYY(22-36) (SEQ. ID. NO. 21)	0.22
906	N-α-Ac-[Bip^{27}]PYY(22-36) (SEQ. ID. NO. 22)	4.46
911	N-α-Ac-[Nal^{27}]PYY(22-36) (SEQ. ID. NO. 23)	0.39
915	N-α-Ac-[Trp^{27}]PYY(22-36) (SEQ. ID. NO. 5)	0.10
916	N-α-Ac-[Thi^{27}]PYY(22-36) (SEQ. ID. NO. 6)	0.095
914	N-α-Ac-[Phe^{27}]PYY(25-36) (SEQ. ID. NO. 26)	0.15
913	N-α-Ac-[Tic^{27}]PYY(22- 36) (SEQ. ID. NO. 25)	4.50

NPY/PYY receptors characterized to date have been broadly classified into Y-1, Y-2 and Y-3 subtypes (Balsubramaniam et al. *J. Biol. Chem.* 265:14724, 1990; Michel, *Trends Pharmacol. Sci.* 12:389, 1991). Both Y-1 and Y-2 receptors exhibit a preference for PYY over NPY, and more significantly C-terminal fragments of NPY and PYY are effective only at the Y-2 subtype. Y-3 receptors, on the other hand, exhibit a greater affinity for NPY than PYY. Since rat jejunal mucosa antisecretory responses show an order of agonist potency PYY (SEQ. ID. NO. 1)> NPY (SEQ. ID. NO. 24) > PYY(13-36)(SEQ. ID. NO. 32) > NPY(13-36)(SEQ. ID. NO. 33) these epithelial receptors are Y-2 like, and are completely insensitive to the Y-1 selective agonist [Pro^{34}]NPY (Cox et al. *Peptides*, *supra*). The results further describe N-α-Ac-PYY(22-36)

(SEQ. ID. NO. 14) and N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3) to be more potent than PYY(22-36)(SEQ. ID. NO. 10) and the corresponding C-terminal fragments of NPY of varying lengths (Cox et al. *Br. J. Pharmacol. supra*). The higher affinity for PYY (SEQ. ID. NO. 1) and its C-terminal fragments compared with NPY (SEQ. ID. NO. 24) and its respective fragments is in agreement with the order of potency obtained from receptor binding studies with rat intestinal epithelial membranes (Laburthe et al. *supra*; Laburthe, *supra*; Voisin et al. *Ann. N.Y. Acad. Sci. supra*; Voisin et al. *Am. J. Physiol.*)

In addition, analogs listed in Table 3 were synthesized as described above and tested for binding activity. The results shown in Table 3 demonstrate that N-α-Ac-[Tyr^{22}, Phe^{27}]PYY(22-36) (SEQ. ID. NO. 7) is similar in its competitive binding as PYY (SEQ. ID. NO. 1), indicating that the introduction of an aromatic amino acid, e.g., Tyr, at position 22 is an effective PYY analog.

TABLE 3

PEPTIDE NO.	Peptide Structure	IC_{50} (nM)
	PYY (SEQ. ID. NO. 1)	0.10
917	N-α-Ac-[Phe^{27}, Thi^{36}]PYY(22-26) (SEQ. ID. NO. 27)	4.46
918	N-α-Ac-[Thz^{26}, Phe^{27}]Pyy(22-36) (SEQ. ID. NO. 28)	4.50
904	N-α-Ac-[Pcp^{27}]PYY(22-36) (SEQ. ID. NO. 29)	1.58
908	N-α-Ac-[$Phe^{22,27}$]PYY(22-36) (SEQ. ID. NO. 30)	11.22
910	N-α-Ac-[Tyr^{22}, Phe^{27}]PYY(22-36) (SEQ. ID. NO. 7)	0.10

USE

In the practice of the method of the present invention, an effective amount of an any one or combination of the analogs of the invention, e.g., N-α-Ac-[Phe^{27}]PYY(22-36)(SEQ. ID. NO. 3), N-α-Ac-[Trp^{27}]PYY(22-36)(SEQ. ID. NO. 24), N-α-Ac-[Phe^{27}]PYY(25-36)(SEQ. ID. NO. 3), N-α-Ac-[Thi^{27}]PYY(22-36) (SEQ. ID. NO. 6) or derivative thereof, is administered via any of the usual and acceptable methods known in the art, either singly or in combination with another compound or compounds of the present invention. These compounds or compositions can thus be administered orally (e.g., buccal cavity), sublingually, parenterally (e.g., intramuscularly, intravenously, or subcutaneously), rectally (e.g., by suppositories or washings), transdermally (e.g., skin electroporation) or by inhalation (e.g., by aerosol), and in the form or either solid, liquid or gaseous dosage, including tablets and suspensions. The administration can be conducted in a single unit dosage form with continuous therapy or in a single dose therapy *ad libitum*.

Thus, the method of the present invention is practiced when relief of symptoms is specifically required or perhaps imminent. Alternatively, the method of the present invention is effectively practiced as continuous or prophylactic treatment.

Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids or gases; thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (e.g. binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, aerosols, and the like. The carrier can be selected from the various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulation for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, talc, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers and the like. Suitable pharmaceutical carriers and their formulation are described in *Remington's Pharmaceutical*

Sciences by E.W. Martin. Such compositions will, in any event, contain an effective amount of the active compound together with a suitable carrier so as to prepare the proper dosage form for proper administration to the recipient.

The dose of the compound of the present invention for treating the above-mentioned disorders varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such amount of the active compound as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount". Thus, a typical administration is oral administration or parenteral administration. The daily dose in the case of oral administration is typically in the range of 0.1 to 100 mg/kg body weight, and the daily dose in the case of parenteral administration is typically in the range of 0.001 to 50 mg/kg body weight.

To be effective for the prevention or treatment of gastroenterological disorders, especially infectious (e.g. viral or bacterial) or inflammatory diarrhea, or diarrhea resulting from surgery, it is important that the therapeutic agents be relatively non-toxic, non-antigenic and non-irritating at the levels in actual use.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Claims:

1. A compound having the formula:

$$
\begin{array}{l}
R_1 \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad R_3 \\
\quad\backslash \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad / \\
R_2\text{-}X\text{-}A^{22}\text{-}A^{23}\text{-}A^{24}\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}Y\text{-}R_4
\end{array}
$$

wherein

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R_1 and R_2;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to R_3 and R_4;

R_1 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

R_2 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^{22} is an aromatic amino acid, Ala, Aib, Anb, N-Me-Ala, or is deleted;

A^{23} is Ser, Thr, Ala, Aib, N-Me-Ser, N-Me-Thr, N-Me-Ala, D-Trp, or is deleted;

A^{24} is Leu, Gly, Ile, Val, Trp, Aib, Anb, N-Me-Leu, or is deleted;

A^{25} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight hain C_1-C_{10} alkyl group, or an aryl group), Orn or is deleted;

A^{26} is Ala, His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched chain or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn,or is deleted;

A^{27} is an aromatic amino acid other than Tyr;

A^{28} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{29} is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A^{30} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;
A^{31} is Val, Ile, Trp, Aib, Anb, or N-Me-Val;
A^{32} is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;
R_3 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl; and
R_4 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein A^{27} is Phe, Nal, Bip, Pcp, Tic, Trp, Trp, Bth, Thi, or Dip.

3. The compound of claim 1, where X is A^{17}-A^{18}-A^{19}-A^{20}-A^{21} wherein
A^{17} is Cys, Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A^{18} is Cys, Ser, Thr, N-Me-Ser, or N-Me-Thr;
A^{19} is Cys, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), or Orn;
A^{20} is an aromatic amino acid or Cys; and
A^{21} is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof.

4. The compound of claim 1, where Y is A^{33}-A^{34}-A^{35}-A^{36} wherein

A^{33} is Cys, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), or Orn;

A^{34} is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Aib, or Anb;

A^{35} is Cys, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), or Orn; and

A^{36} is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof.

5. The compound of claim 4, wherein said compound has the formula: N-α-Ac-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 3), or a pharmaceutically acceptable salt thereof.

6. The compound of claim 4, wherein said compound has the formula: H-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 4), or a pharmaceutically acceptable salt thereof.

7. The compound of claim 4, wherein said compound has the formula: N-α-Ac-Ala-Ser-Leu-Arg-His-Trp-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 5), or a pharmaceutically acceptable salt thereof.

8. The compound of claim 4, wherein said compound has the formula:
N-α-Ac-Ala-Ser-Leu-Arg-His-Thi-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 6), or a pharmaceutically acceptable salt thereof.

9. The compound of claim 4, wherein said compound has the formula:
N-α-Ac-Tyr-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 7), or a pharmaceutically acceptable salt thereof.

10. A compound having the formula:

$$\begin{array}{l}
\;R_1 \hspace{17em} R_3 \\
\quad \backslash \hspace{17em} / \\
R_2\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}Y\text{-}R_4
\end{array}$$

wherein

the N-terminal amino acid is bonded to R_1 and R_2;

Y is a chain of 0-4 amino acids, inclusive the C-terminal one of which is bonded to R_3 and R_4;

R_1 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

R_2 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^{25} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn or is deleted;

A^{26} is Ala, His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or an aryl group), Orn or is deleted;

A^{27} is an aromatic amino acid;

A^{28} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{29} is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A^{30} is Leu, Ile, Val, Trp, Aib, Anb, or N-Me-Leu;

A^{31} is Val, Ile, Trp, Aib, Anb, or N-Me-Val;

A^{32} is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;

R_3 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl; and

R_4 is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl or C_7-C_{18} alkaryl, or a pharmaceutically acceptable salt thereof.

11. The compound of claim 10, wherein A^{27} is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

12. The compound of claim 10, wherein Y is A^{33}-A^{34}-A^{35}-A^{36} wherein

A^{33} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), Cys, or Orn;

A^{34} is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Alb, or Anb;

A^{35} is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or C_6-C_{18} aryl group), Cys, or Orn; and

A^{36} is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof.

13. The compound of claim 12, wherein said compound has the formula:
N-α-Ac-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2 (SEQ. ID. NO. 26), or a pharmaceutically acceptable salt thereof.

14. A therapeutic composition capable of decreasing excess intestinal water and electrolyte secretion, said composition comprising a therapeutically effective amount of the compound of claim 1 and claim 10, together with a pharmaceutically acceptable carrier substance.

15. A method of decreasing excess intestinal water and electrolyte secretion in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 14.

16. A method of regulating cell proliferation in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 14.

17. A method of augmenting nutrient transport in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 14.

18. A method or regulating lipolysis in a mammal, said method comprising adminsitering to said mammal a therapeutically effective amount of the composition of claim 14.

19. A method of regulating blood flow in a mammal, said method comprising adminsitering to said mammal a therapeutically effective amount of the composition of claim 14.

20. A dimeric compound comprising either two peptides of claim 10, or one peptide of claim 1 or one peptide of claim 10, wherein said dimer is formed by either an amide bo, or a disulfide bridge between said two peptides.

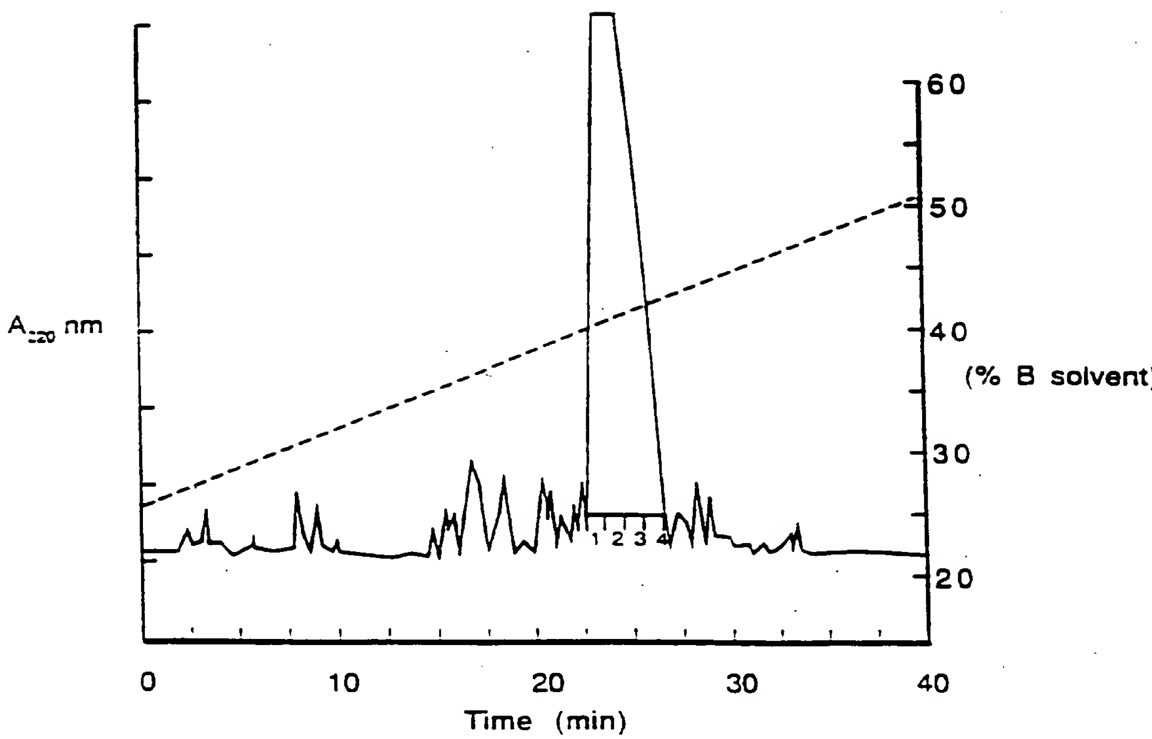
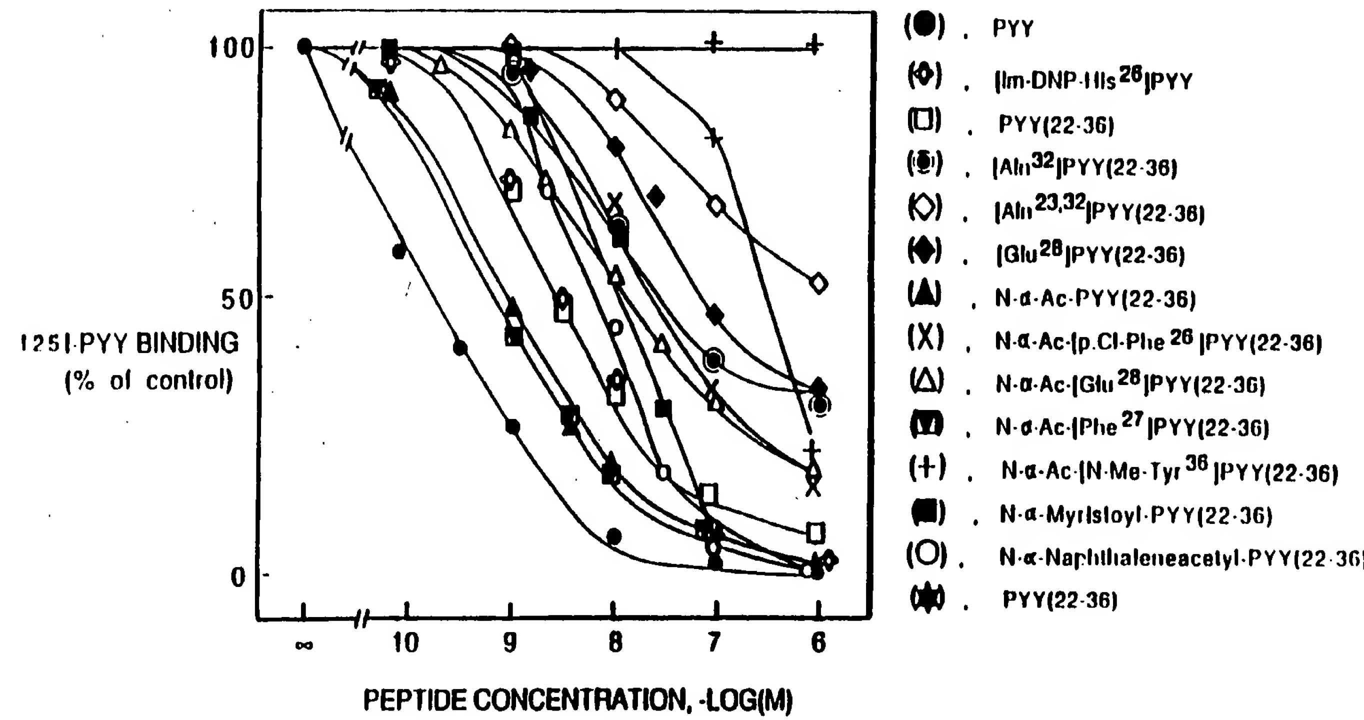


FIG. 1


100
50
0
125I-PYY BINDING
(% of control)
∞
10
9
8
7
6
PEPTIDE CONCENTRATION, -LOG(M)
PYY
[Im-DNP-His26]PYY
PYY(22-36)
[Aib32]PYY(22-36)
[Aib23,32]PYY(22-36)
[Glu28]PYY(22-36)
N-α-Ac-PYY(22-36)
N-α-Ac-[p.Cl-Phe26]PYY(22-36)
N-α-Ac-[Glu28]PYY(22-36)
N-α-Ac-[Phe27]PYY(22-36)
N-α-Ac-[N-Me-Tyr36]PYY(22-36)
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N-α-Naphthaleneacetyl-PYY(22-36)
PYY(22-36)

FIG. 2

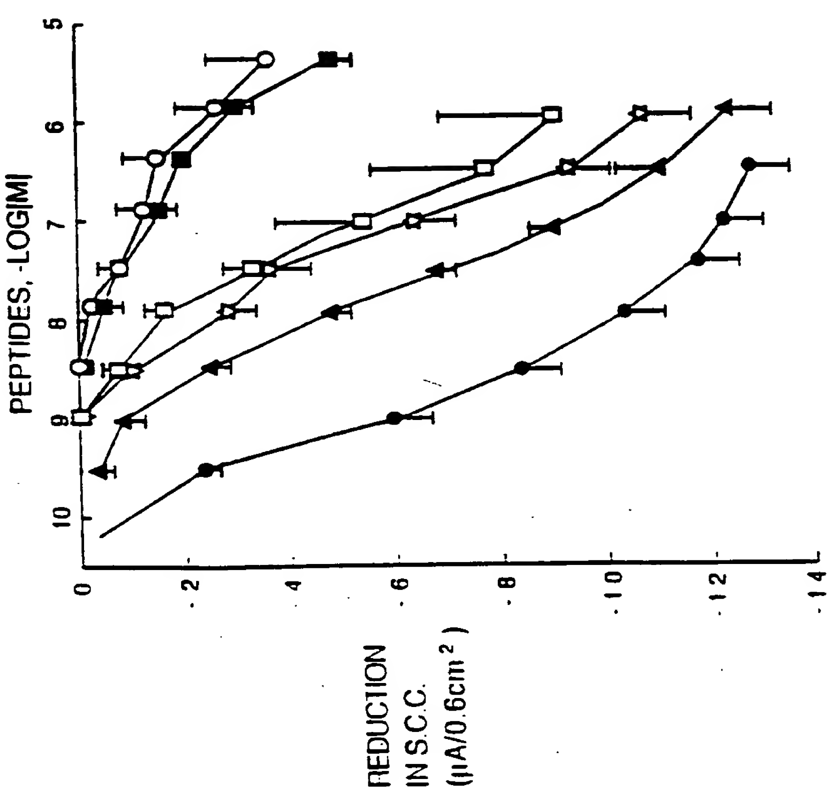
PEPTIDES, -LOG[M]
10
9
8
7
6
5
0
-2
-4
-6
-8
-10
-12
-14
REDUCTION
IN S.C.C.
(µA/0.6cm²)

FIG. 3A

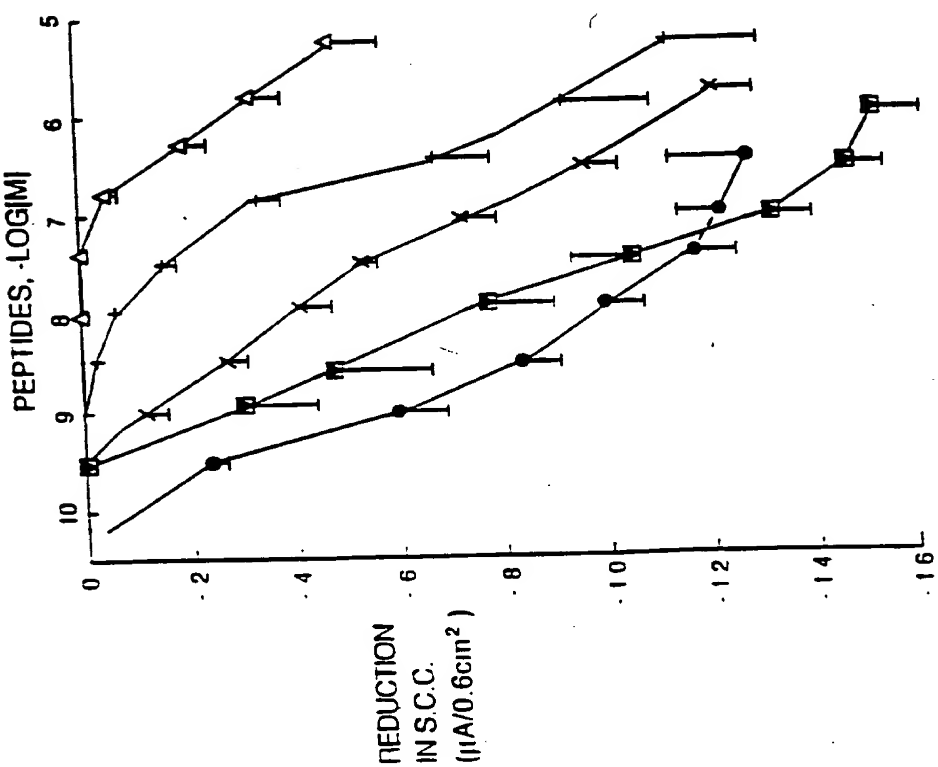
PEPTIDES, -LOG[M]
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
REDUCTION
IN S.C.C.
(µA/0.6cm²)

FIG. 3B

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US94/03380

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/16, 37/02; C07K 5/00, 7/00, 15/00, 17/00

US CL :514/12, 13, 14, 15, 16, 17; 530/324, 325, 326, 327, 328, 329

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 13, 14, 15, 16, 17; 530/324, 325, 326, 327, 328, 329

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,026,685 (BOUBLIK ET AL) 25 June 1991, see col. 3.	1-2
X	Chem. Pharm Bull., Volume 36, No. 7, issued 1988, T. Ishiguro et al, "Synthesis of Peptide Fragments of Neuropeptide Y: Potent inhibitors of Calmodulin-stimulated phosphodiesterase", pages 2720-2723, especially table II.	1, 19
X	J. Med. Chem, Volume 35, issued 1992, Feinstein et al, "Structural Requirements for Neuropeptide Y^{18-36}-Evoked Hypotension: A Systematic Study", pages 2836-2843, especially compound number 24 in Table I.	1-2

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 JUNE 1994	JUN 14 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SHEELA J. HUFF *D. Kuyza for*
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Peptides, Volume 14, issued 1993, A Balasubramaniam et al. "Structure-Activity Studies of Peptide YY(22-36): N-alpha-Ac-[Phe^{27}]PYY(22-36), a Potent Antisecretory Peptide in Rat Jejunum", pages 1011-1016, especially Table I.	1-5
X	JP, A, 64-6294 (ISHIGURO ET AL) 01 October 1989, see pages 1202, 1204, 1209-1210, 1219, 1221.	1, 16, 19

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

USPTO APS
search terms: neuropeptude Y, peptide yy, leu-val-thr-arg-gln-arg, leu-val-ala-arg-gln-arg, leu-val-trp-arg-gln-arg, (phe or nal or trp or bip or pcp or tic or bth or thi or dip)(W)(leu or ile or val or trp or aib or anb)(W)(asn or ala or gln or gly or trp))(W)(leu or ile or val or trp or aib or anb)(W)(val or ile or trp or aib or anb)

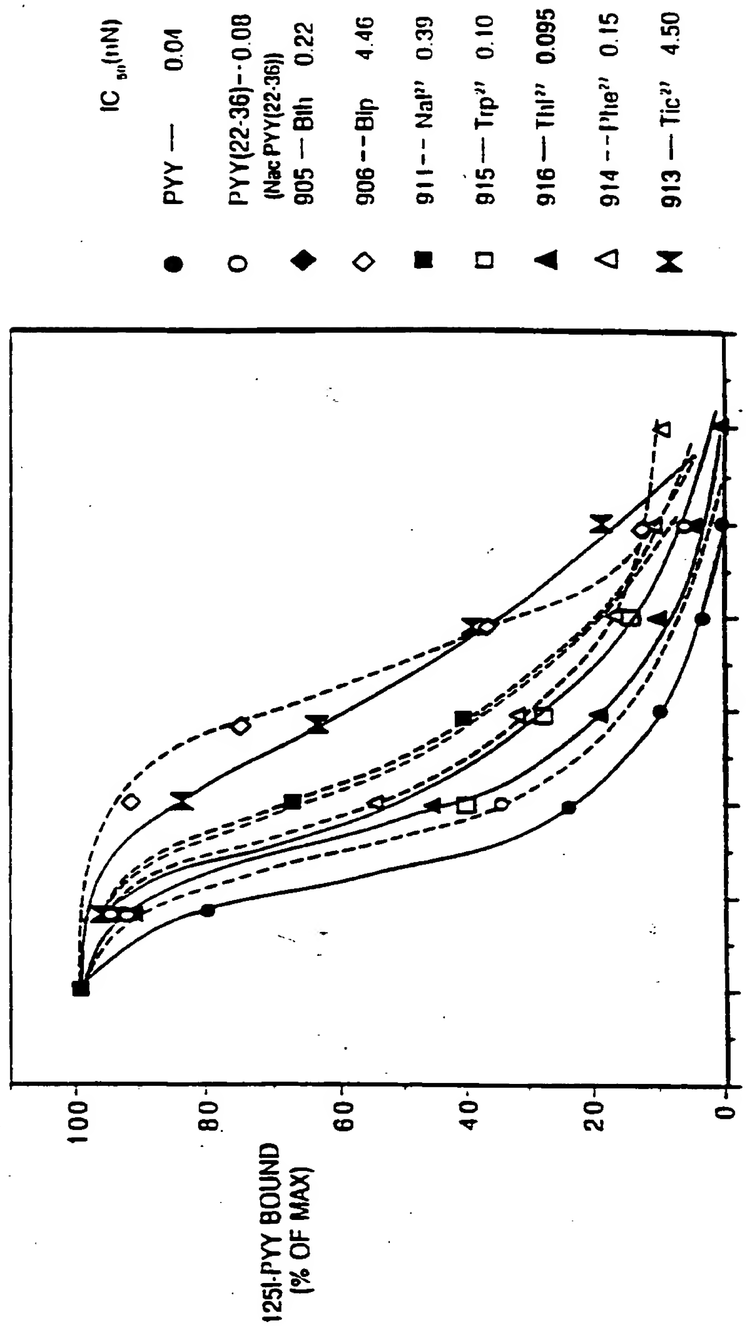
IC50(nM)
PYY — 0.04
PYY(22-36) -- 0.08
(Nac PYY(22-36))
905 — Bth 0.22
906 -- Bip 4.46
911 -- Nal27 0.39
915 — Trp27 0.10
916 — Thi27 0.095
914 -- Phe27 0.15
913 — Tic27 4.50
125I-PYY BOUND
(% OF MAX)
100
80
60
40
20
0
-∞
-11
-10
-9
-8
-7
-6
PEPTIDES CONCENTRATION, -LOG(M)

FIG. 4